Figure 1

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                    ....|....| ....|....| ....|....| ....|....| ....|....|
                          10         20         30         40         50
Hordeum vulgare     ---MASD-HR RFVLSGAVLL SVLAVAAATL E--------- SVKDECQLGV
Oryza sativa        ---MASN-KV VFSVLLLAVV SVLAATATMA EYHHQDQVVY TPGPLCQPGM
Hordeum spontaneum  ---MAFK--Y QLLLSAAVML AILAATVT-- ---------- SFGDMCAPGD
Eleusine coracana   ---------- ---------- ---------- ---------- SVGTSCIPGM
Secale cereale      ---------- ---------- ---------- ---------- SVGGQCVPGL
Triticum durum      ---MACKSSC SLLLLAAVLL SVLAA--A-- ---------- SASGSCVPGV
Zea mays            MASSSSSSHR RLILAAAVLL SVLAAASA-- ---------- SAGTSCVPGW
Tritcum aestivum    ---MASN-HR RFLLSGAVLL SVLAAVAA-L E--------- SVEDECQPGV

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                          60         70         80         90        100
Hordeum vulgare     DFPHNPLATC HTYVIKRVCG ---RGPSRPM LV-------- ------KERC
Oryza sativa        GYPMYPLPRC RALVKRQCVG ---RGTAAAA EQ-------- -----VRRDC
Hordeum spontaneum  ALPANPLRAC RTYVVSQIC- --HVGPRLST WD-------- -----MKRRC
Eleusine coracana   AIPHNPLDSC RWYVAKRACG ---VGPRLAT QE-------- -----MKARC
Secale cereale      AMPHNPLGAC RTYVVSQIC- --HVGPRLFT WD-------- -----MKRRC
Triticum durum      AFRTNLLPHC RDYVLQQTCG TFTPGSKLPE WMTSASIYSP GKPYLAKLYC
Zea mays            AIPHNPLPSC RWYVTSRTCG ---IGPRLPW PE-------- -----LKRRC
Tritcum aestivum    AFPHNALATC HTYVIKRVCG ---RGPSRPM LV-------- ------KERC

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                         110        120        130        140        150
Hordeum vulgare     CRELAAVP-D HCRCEALRIL MDGVRTPE-- ----GRVVEG RLGDRRDCPR
Oryza sativa        CRQLAAVDDS WCRCEAISHM LGGIYRELG- ---APDVGHP MSEVFRGCRR
Hordeum spontaneum  CDELSAIP-A YCRCEALRII MDGTVTWQ-- ----GVFG-A YFKDMPNCPR
Eleusine coracana   CRQLEAIP-A YCRCEAVRIL MDGVVTP--- ---SGQHEGR LLQDLPGCPR
Secale cereale      CDELLAIP-A YCRCEALRIL MDGVVTQQ-- ----GVFEGG YLKDMPNCPR
Triticum durum      CQELAEIS-Q QCRCEALRYF IALPVPSQPV DPRSGNVGES GLIDLPGCPR
Zea mays            CRELADIP-A YCRCTALSIL MDGAIPPGP- ---DAQLEGR -LEDLPGCPR
Tritcum aestivum    CRELAVVP-D YCRCEALRVL MDGVRAEE-- ----GHVVEG RLGDRRDCPR

                    ....|....| ....|....| ....|....| ....|....| ...
                         160        170        180        190
Hordeum vulgare     EEQRAFAATL VTAAECNLSS VQAPGVRLVL LADG------ ---
Oryza sativa        GDLERAAASL PAFCNVDIPN GGG-GVCYWL ARSGY----- ---
Hordeum spontaneum  VMQTSYAANL VNPQECNLWT IHGSPSCPEL QPGYEVVL-- ---
Eleusine coracana   QVQRAFAPKL VTEVECNLAT IHGGPFCLSL LGAGE----- ---
Secale cereale      VTQRSYAATL VAPQECNLPT IHGSPYCPTL QAGY------ ---
Triticum durum      EMQWDFVRLL VAPGQCNLAT IHNVRYCPAV EQPLWI---- ---
Zea mays            EVQRGFAATL VTEAECNLAT ISGVAECPWI LGGGTMPSK- ---
Tritcum aestivum    EAQREFAATL VTAAECNLPT VS--GVGSTL GATGRWMTIE LPK
```

Figure 2

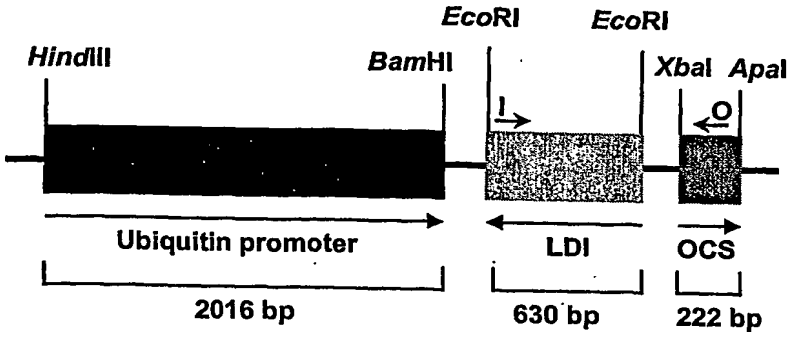


Figure 3

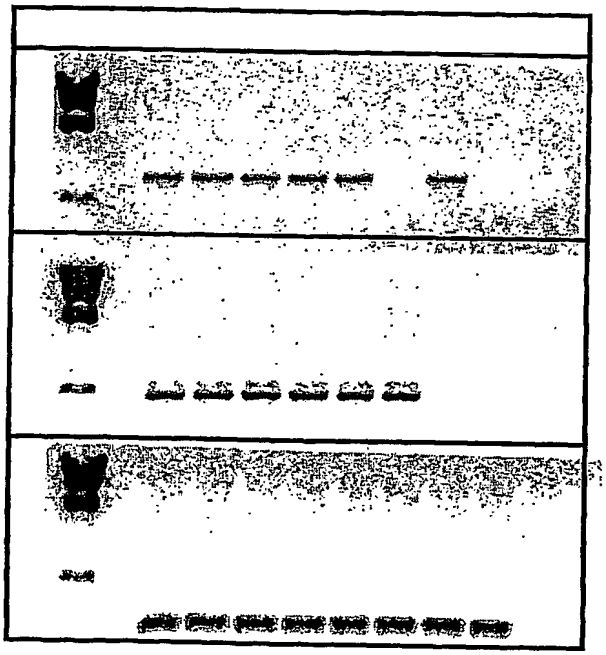
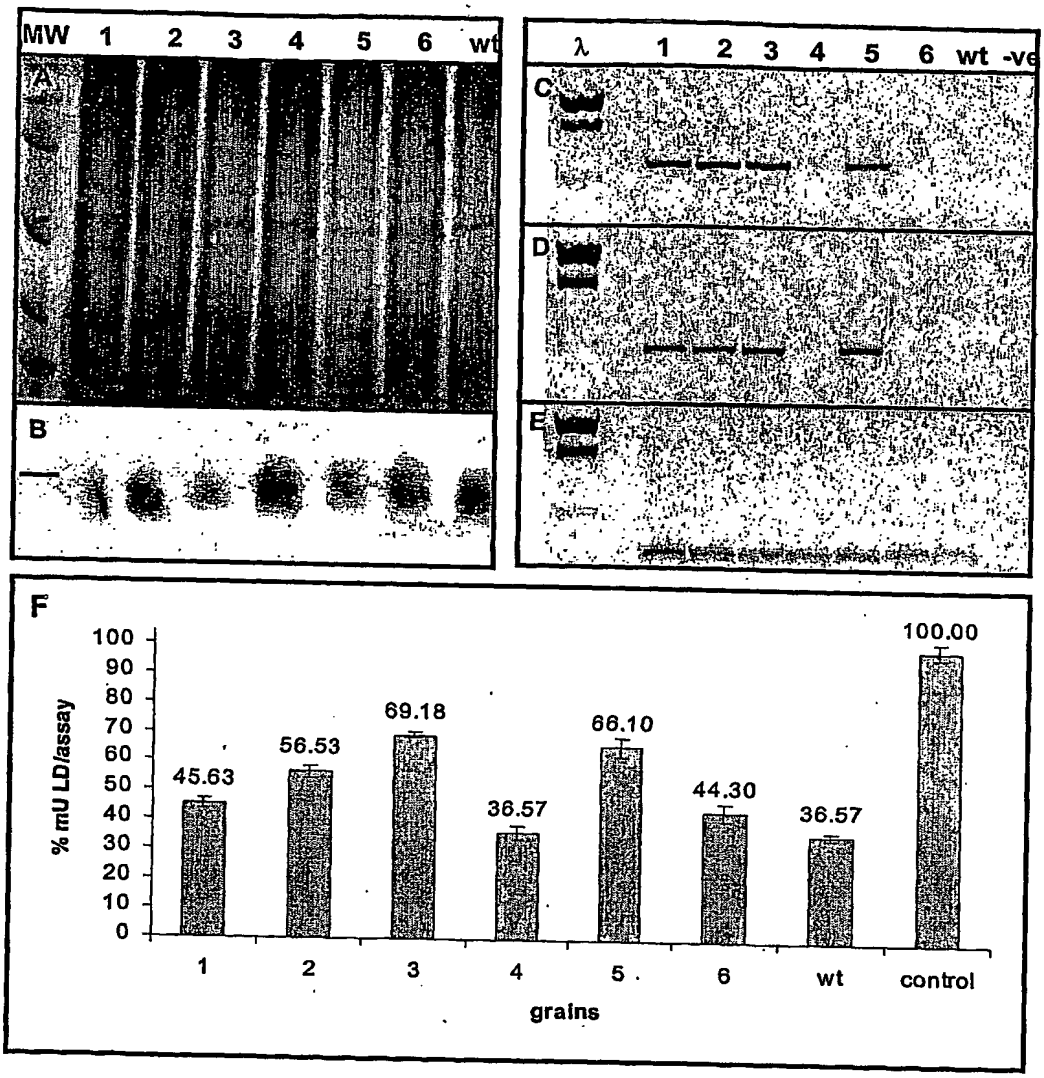


Figure 4



(A) 10% SDS-PAGE of LDI extracts corresponding to 5 μg protein.
(B) Immunoblot developed with antiserum against LDI; bar represents 21.5 kD.
(C) PCR of genomic DNA with primers Inhib-6 + OCS-II for the *LDI* gene in antisense direction (817 bp).
(D) PCR of genomic DNA primers BAR-I + BAR-II for the *bar* gene (534 bp).
(E) PCR of genomic DNA primers TUB-F+ TUB-R for the *tubulin* gene (217 bp).
(F) LDI activity assay. The control represents the amount of LD used for each assay. LDI extracts corresponding to 10 μg protein were mixed with LD and assayed for remaining LD activity. Each value represents the mean ± SE of three replicate experiments.

1-6: six individual grains; **wt:** wildtype; **MW:** molecular weight marker sizes in kD are 97.4, 66.2, 45, 31, 21.5 and 14.4; λ: λ/*Hind* III molecular weight marker; **-ve:** negative control of PCR.

Figure 5

(A) 12% SDS-PAGE of LDI extracts corresponding to 5µg protein.
(B) Immunoblot developed with antiserum against LDI; bar represents 21.5 kD.

U3: homozygous T_2 generation transgenic line U3; **U4**: homozygous T_2 generation transgenic line U4; **wt**: wildtype; **MW**: molecular weight marker sizes in kD are 97.4, 66.2, 45, 31, 21.5 and 14.4.

Figure 6

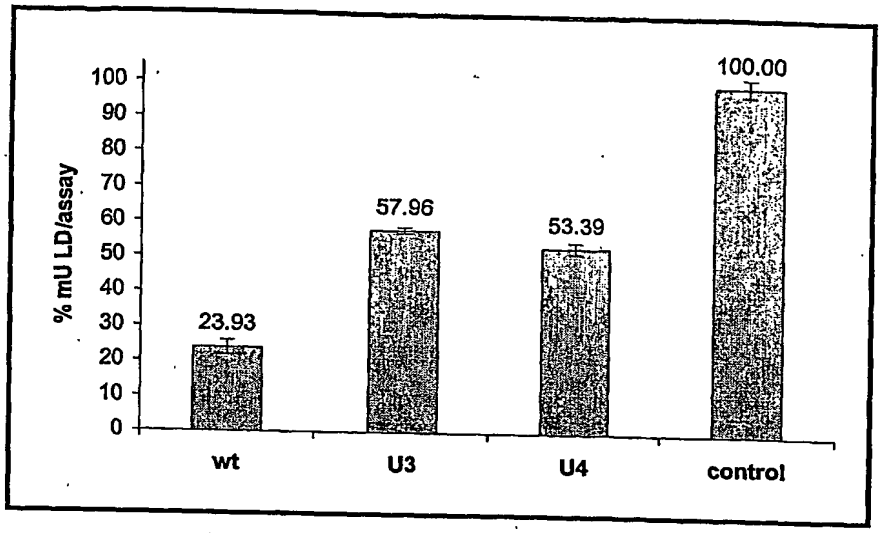
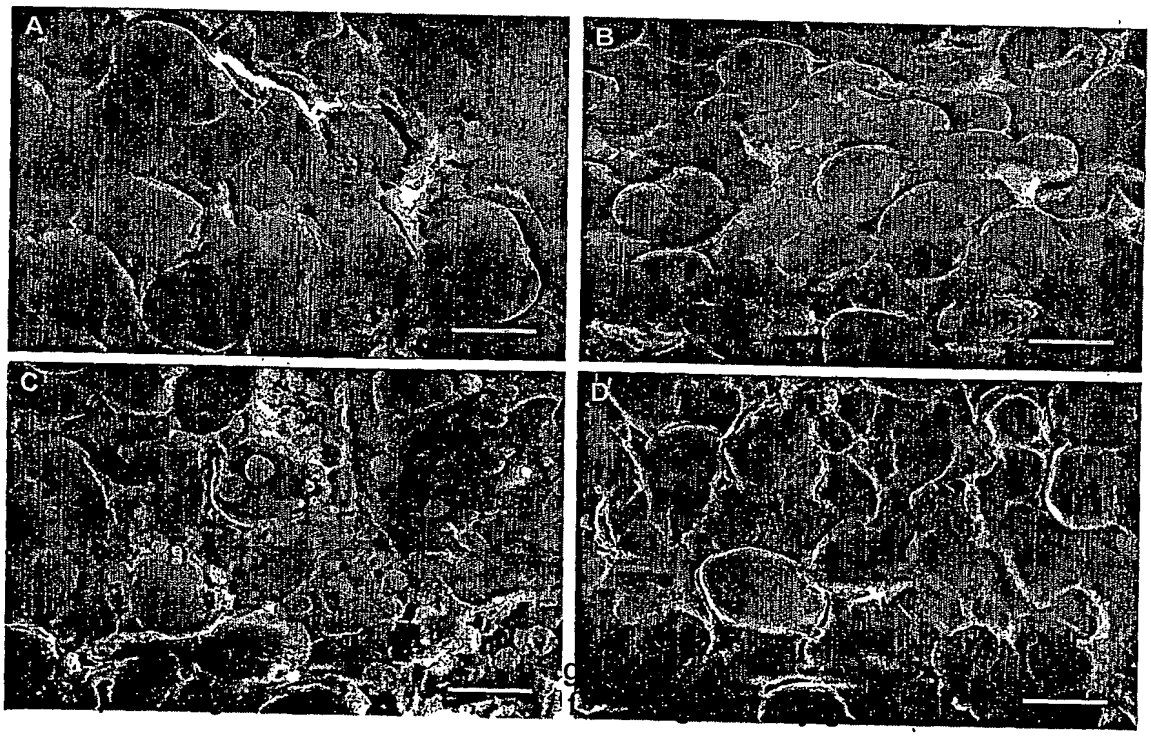


Figure 7



(A) wildtype barley grain.
(B) transgenic T_2 generation grain of homozygous line U4.
(C) wildtype T_1 grain of heterozygous transgenic line U3.
(D) transgenic T_1 grain of heterozygous line U3.
Bar is 10 μm long.

Figure 8

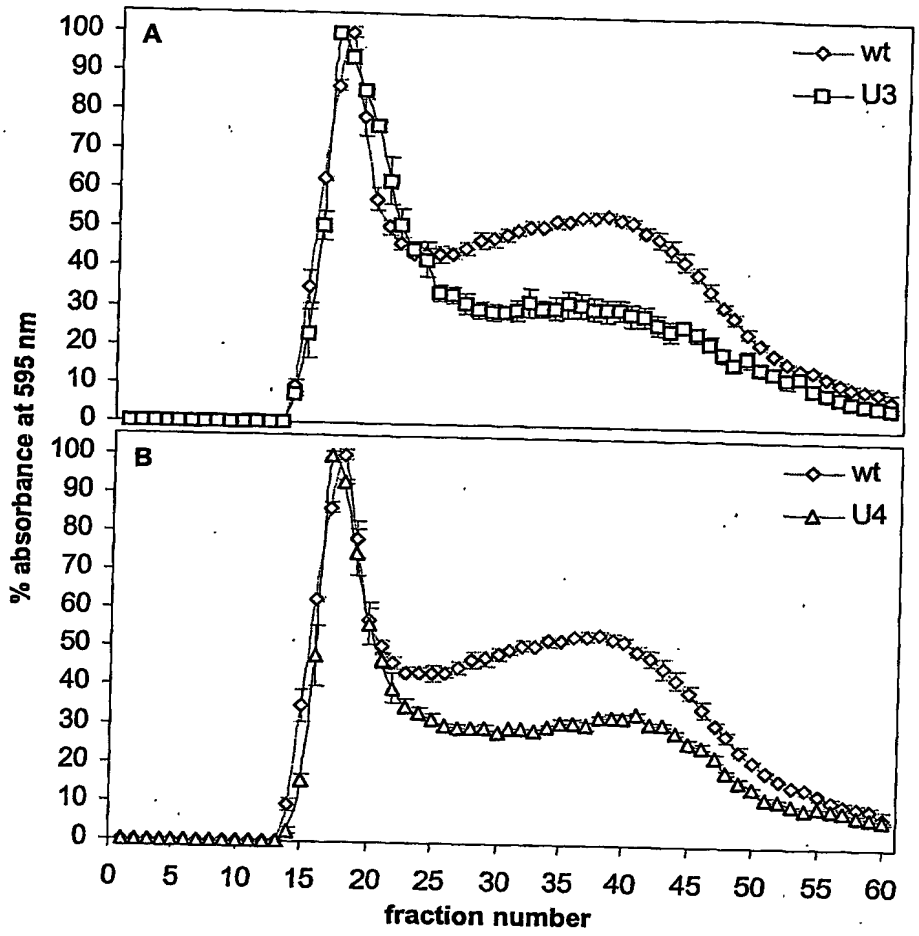

A
wt
U3
B
wt
U4
% absorbance at 595 nm
fraction number
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30
35
40
45
50
55
60

Figure 9

DNA % Identity						
	Hordeum vulgare SEQ. ID. No. 3	*Hordeum vulgare SEQ. ID. No. 1*	*Triticum aestivum*	*Zea mays*	*Oryza sativa*	*Hordeum sponteneum*
Hordeum vulgare SEQ. ID. No. 3	100	76.34				
Hordeum vulgare SEQ. ID. No. 1	98.84	100	77.27	63.45	42.35	43.09
Triticum aestivum			100	65.38	45.57	46.05
Zea mays				100	41.37	46.81
Oryza sativa					100	49.28
Hordeum sponteneum						100

% amino acid identities								
	Hordeum vulgare SEQ. ID. No. 4	*Hordeum vulgare SEQ. ID. No. 2*	*Hordeum sponteneum*	*Oryza sativa*	*Triticum aestivum*	*Zea mays*	*Secale cereale*	*Eleusine coracana*
Hordeum vulgare SEQ. ID. No. 4	100							
Hordeum vulgare SEQ. ID. No. 2	98.64	100	42.86	34.01	80.95	53.06	40.14	42.18
Hordeum sponteneum		43.54	100	29.92	43.54	48.98	66.67	46.26
Oryza sativa		33.75	25.63	100	31.88	33.13	23.75	25
Triticum aestivum		78.43	41.83	33.33	100	54.25	39.22	39.22
Zea mays		50.97	46.45	35.48	53.55	100	43.87	52.9
Secale cereale		48.36	79.51	29.51	50.82	55.74	100	62.3
Eleusine coracana		51.22	54.47	29.27	50.41	66.67	61.79	100